

## ORIGINAL PAPERS

# Outcome of Children with Acute Lymphoblastic Leukemia Treated with the ALL-BFM 95 Protocol during 2001-2014 at the Pediatric Hematology Center from Tîrgu-Mures

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## Abstract

Survival in childhood acute lymphoblastic leukemia (ALL) has improved substantially during the last years. The aim of the paper is to present the outcome of patients with ALL treated with the ALL-BFM 95 protocol between 2001-2014 in the Pediatric Clinic from Tîrgu-Mures. Eighty-eight newly diagnosed patients with ALL were assessed retrospectively, retrieving data from the clinical records. Kaplan-Meier survival curves and log-rank analysis were performed with SPSS program. ALL appeared more frequently in males (60,2%) and in the age group 2-5 years (44%). The overall survival (OS) was 72% and the 5-year survival rate of 67,65%. Thirty-two patients had molecular biology tests performed from the bone marrow at diagnosis and two cases had the minor BCR-ABL1 rearrangement. The outcome of the 15 patients with CNS involvement at diagnosis was significantly lower (35,71%) compared to patients without CNS involvement (80,60%) ( $p=0.0003$ ). Survival rate was higher in precursor B ALL than in T-cell ALL (76,92% versus 66,67%;  $p=0.0004$ ). Patients with M1 type BM on the 33<sup>rd</sup> day ( $n=72$ ) had an OS of 79,17% compared to the 5 patients with M2 type BM whose OS was only 20% ( $p=0.0127$ ). **Conclusions:** Results are improving, yet further efforts are needed to improve outcome.

**Keywords:** acute lymphoblastic leukemia, child, survival, risk factors

## Rezumat

Supraviețuirea în leucemia acută limfoblastică (LAL) la copil s-a ameliorat substanțial în ultima perioadă. Scopul lucrării este prezentarea rezultatelor obținute în tratamentul copiilor cu leucemie acută limfoblastică, cu protocolul ALL-BFM 95 în perioada 2001-2014 în centrul de hemato-oncologie pediatrică din Tîrgu-Mureș. S-a efectuat un studiu retrospectiv, analizând datele a 88 de bolnavi cu LAL din foile de observație. Curbele de supraviețuire Kaplan-Meier și analiza log-rank au fost efectuate cu programul statistic IBM SPSS. LAL a apărut mai frecvent la băieți (60,2%) și la grupa de vârstă de 2-5 ani (44%). Supraviețuirea a fost de 72%, iar rata de supraviețuire la 5 ani de 67,65%. Teste de biologie moleculară din măduva osoasă au fost efectuate la 32 de bolnavi dintre care în 2 cazuri s-a detectat rearanjamentul genic BCR-ABL1 minor. Supraviețuirea bolnavilor ( $n=15$ ) care au avut afectarea sistemului nervos central (SNC) la debut, a fost semnificativ mai mică (35,71%) față de bolnavii fără afectare SNC (80,60%) ( $p=0.0003$ ). Rata de supraviețuire a fost mai înaltă la bolnavii cu LAL precursor-B față de cei cu LAL cu celule T (76,92% versus 66,67%;  $p=0.0004$ ). Pacienții ( $n=72$ ) care în ziua 33 de tratament au avut un rezultat de măduvă osoasă de tip M1 au avut o supraviețuire de 79,17% față de cei 5 bolnavi cu măduvă de tip M2 a căror supraviețuire a atins doar 20% ( $p=0.0127$ ). **Concluzii:** Rezultatele au o tendință de îmbunătățire dar sunt necesare eforturi suplimentare pentru a crește rata de supraviețuire în LAL la copil.

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## INTRODUCTION

Acute lymphoblastic leukemia (ALL) is responsible for 25% of all childhood cancers. The outcome has dramatically changed during the last decades, reaching an overall survival rate of approximately 80% in high-income countries and lower rates in low and middle-income countries<sup>1-6</sup>. Recent trials with risk stratification based on biological features of leukemic cells and response to therapy report an even better survival rate of 90%<sup>7</sup>. Experience has shown that the current knowledge about childhood leukemia and its improved outcome is related to cooperative clinical research<sup>8</sup>. Correct diagnosis, risk-stratification, treatment intensity adapted to the risk group and molecular response to treatment, adequate supportive care are needed for best results.

The aim of the paper is to assess the survival rate of ALL in children aged 0-18 years, treated at the Pediatric Clinic from Targu-Mures, Romania during January 2001 and July 2014, making corroborations with prognostic factors such as patient characteristics (age, WBC count at diagnosis, central nervous system (CNS) involvement, testicular involvement, gender) leukemic cell characteristics (FAB morphology, immunophenotype, cytogenetic/genomic alterations) and response to initial treatment (absolute lymphoblast count from peripheral blood after 7 days of steroid therapy, bone marrow (BM) on 15 and 33 days of treatment).

## MATERIAL AND METHODS

Eighty-eight patients aged 0-18 years with ALL were treated during January 2001 and July 2014 at the Pediatric Clinic from Targu-Mures with the therapeutic protocol ALL-BFM 95. From the clinical records of the patients we retrieved data about age at diagnosis, gender, WBC at diagnosis, FAB types and immunophenotype of lymphoblasts, cytogenetic/molecular biology features, absolute lymphoblast count after seven days of corticosteroid therapy, BM morphology on the 15<sup>th</sup> and 33<sup>rd</sup> days of treatment, CNS involvement, relapses, survival. Availability of flow cytometry and molecular biology tests has evolved gradually during this time so that only a fraction of the patients have these tests performed. We calculated the Kaplan-Meier survival curve and survival curves related to individual prognostic factors, log-rank analysis, using the SPSS statistical program. We used the risk stratification according to the protocol ALL-BFM 95: standard risk group patients shared the following clinical and biological features: age 1-6 years, WBC  $<20 \times 10^9/L$  at diagnosis, no T- immunology, no t(9;22), no t(4;11), on day 8 less than  $1 \times 10^9/L$  leukemic cells in the peripheral

blood (PB) and complete remission in the BM on day 33 of treatment; medium-risk patients are characterized by no t(9;22), no t(4;11),  $<1 \times 10^9/L$  leukemic cells in the PB, M1 type BM on day 33 and one or more features from the following three: WBC  $>20 \times 10^9/L$  at diagnosis, age under 1 or above 6 years; high-risk patients have at least one criteria from the following:  $>1 \times 10^9/L$  leukemic cells in PB on day 8, no complete remission on day 33, t(9;22), t(4;11). Type M1 BM indicated lymphoblasts  $<5\%$ , the M2 BM 5-25% lymphoblasts and the M3 BM more than 25% lymphoblasts.

## RESULTS

Eighty-eight patients were diagnosed with ALL between January 2001- July 2014 at the Pediatric Clinic from Targu-Mures, with a slight predominance of males (n=53; 60.2%). The peak incidence appeared in the age group of 2-5 years (44%). Forty-two patients (47.7%) had less than  $20 \times 10^9/L$  WBC at diagnosis. The morphology of the BM showed FAB L1 type lymphoblasts in 48 patients (54.5%), L2 in 35 (39.8%) and L3 in 4 patients (4.5%). Flow-cytometry of bone marrow samples revealed 57 cases of precursor-B ALL, 16 of T-cell ALL and three cases of mature-B cell ALL. Molecular biology investigation was performed in 32 patients with detection of minor and major BCR-ABL1 gene rearrangements. Two patients were positive for minor BCR-ABL1. CNS involvement at diagnosis was present in 14 patients. After seven days of prednisone and one intrathecal administration of methotrexate, 66 patients out of 88 had  $<1 \times 10^9/L$  leukemic cells in PB. Morphology of the BM on the 15<sup>th</sup> day of chemotherapy showed M1 type marrow in 52 patients (61.2%), type M2 in 20 (23.5%) and type M3 in 10 (11.8%) patients. After 33 days of treatment, marrows were type M1 in 76 patients (86.4%) and type M2 in 5 patients (5.7%). Twenty-three patients died from the cohort (28%), the overall survival (OS) rate was 72%. Fifty-three patients were assigned to the standard risk (SR) + medium-risk (MR) group and 29 to the high-risk (HR) group. In the SR + MR group, survival reached 84.62% compared to the HR group with 48.28% OS. OS corroborated with WBC at diagnosis showed a difference, which was not statistically significant: the group with less than  $20 \times 10^9/L$  WBC at diagnosis had an OS of 81.58% versus the group with higher WBC with OS of 63.64%. (p= 0.081). Patients with FAB L1 morphology had an OS rate of 81.40%, those with L2 type an OS of 70.59% and none of the patients with L3 type survived. Among the patients with precursor B-ALL 76,92% survived, compa-

red to T-cell ALL (66.67%) and mature B-cell ALL (0.00%). This statistical difference was significant ( $p=0.0004$ ). Out of the 32 molecular biology tests performed from bone marrow samples at diagnosis, two harbored the BCR-ABL1 rearrangement. One of these patients received HR arm regimen and allogeneic stem cell transplantation (ASCT), the other HR regimen plus imatinib mesylate. Both died, the 5-year-old girl at 7 months after ASCT in invasive herpes infection, the 12-year-old boy with poor compliance and metabolic complications (diabetes) died after the 6th HR block in invasive aspergillosis. Fourteen patients had positive CSF at diagnosis, nine of them died. OS was as low as 35.71% in patients with CNS involvement compared to patients without CNS involvement, where OS was 80.60%. CNS involvement turned out to be a poor prognostic factor, the difference in survival rate was significant ( $p=0.0003$ ). OS in good prednisone responders (GPR) was 78.46% whereas in poor prednisone responders (PPR) was 57.14% ( $p=0.1496$ ). The BM results on day 15 of chemotherapy did not show to be significant in survival rate, though differences existed with 78.85% OS in M1 BM type, 70.59% in M2 and 62.50% in M3 types. BM morphology on day 33 of treatment was an important prognostic factor which influenced survival rate significantly: out of the 72 patients with M1 type marrow, the survival rate was 79.17%, compared to an OS of 20% in the five patients with M2 type BM. ( $p=0.0127$ ). Twelve patients relapsed, 2-2 with exclusively BM or CNS relapse and eight with mixed type relapse. OS in these patients was 25% versus non-relapsed patients with OS of 81.16% ( $p=0.0001$ ). Two of the relapsed patients received HSCT, one AuSCT and the other patient underwent ASCT with matched unrelated donor (MUD). Both of them are alive. The 5-year survival rate was 67.65%.

## DISCUSSION

In our cohort of patients, T-cell ALL had a slightly elevated rate (21%) than postulated in the literature (10-15%)<sup>9-11</sup> with an OS of 66.67%. Wei W et al. studied the early treatment responses with the detection of minimal residual disease (MRD) in a group of 74 patients with T-cell ALL and concluded that MRD was the most powerful prognostic factor but also emphasized that conventional morphology assessment of BM at days 15 and 33 may have important roles in predicting outcome and tailoring treatment in countries with limited financial resources for MRD monitoring<sup>12</sup>.

In high-income countries (HIC), the improved diagnosis, risk-stratification, and supportive care along

with personalized treatments based on minimal residual disease, pharmacokinetics and pharmacogenetics have led to cure rates as high as 76-86%<sup>11,13</sup>. Lower survival rates are achieved in low and middle-income countries (LMIC). On a large cohort of Mexican children, treated with a Dana-Farber Cancer Institute Protocol 00-01, Jimenez, et al. report an OS of 63.9% and EFS of 52.3% after an average follow-up of 3.9 years, with more common cause of death being infection<sup>3</sup>. In a cohort of high-risk ALL Chinese children treated with the ALL-BFM 95 protocol the authors noticed an improvement in survival (62% five-year probability of EFS), with better results when hemopoietic stem cell transplantation was provided<sup>14</sup>. In Thailand, 486 patients were treated with the Thai national protocol between 2006-2008, with a 5-year OS of 67.2%, here the socioeconomic factors and patient compliance were the key elements which determined survival rate<sup>4</sup>. This result presents a significant improvement compared to another work which compares survival in ALL patients treated during 1990-2011 in Southern Thailand and the US, where the overall 5-year relative survival was 43% in Thailand and 79% in the US<sup>5</sup>. Cezar R et al. analyzed 33 ALL patients treated in Brasil in 2004-2009, 18 patients were assigned to HR group, 13 patients to MR and 2 patients in SR group. The 6-year OS was  $67.5\% \pm 3.47\%$ <sup>15</sup>. Our study led to comparable results. Causes of poorer survival rates in LMIC are complexes. Better outcomes have been reported when patients were enrolled in clinical trials<sup>16</sup>. The rate of infection-related mortality is more than 10 × higher in LMIC<sup>17</sup>. Practical recommendations have been made by Ceppi F et al. for improving the supportive care of children with ALL in LMIC<sup>18</sup>. International collaborations have proved their role in ameliorating treatment outcomes in childhood ALL, as seen after the results reported from the Intercontinental Trial ALL IC-BFM 2002<sup>9</sup>. Partnerships, technology sharing between sponsoring and partner pediatric oncology centers could be further modalities to improve outcome in children with ALL in LMIC<sup>6</sup>.

Advances made in diagnostics and national and international collaborations led in Czech ALL patients to OS of 84-92%, comparable to leading international study groups<sup>2</sup>.

The AIEOP-BFM ALL 2000 trial compared outcomes using 10 mg/m<sup>2</sup> dexamethasone versus 60 mg/m<sup>2</sup> prednisone in the induction and stated that there was a survival benefit from dexamethasone only for patients with T-cell ALL<sup>19</sup>.

A meta-analysis of 16,623 ALL patients aged 1-18 years showed that with recent treatment protocols, cra-

nial radiotherapy was associated with reduced risk of relapse only in patients who had overt CNS leukemia at diagnosis so that cranial radiotherapy does not have an impact on relapse in children with ALL<sup>20</sup>.

Minimal residual disease (MRD) is the strongest current predictor of outcome and help individualize treatment intensity so that low-risk patients can avoid the toxicity of intensified regimens<sup>6</sup>. Early recovery of the absolute lymphocyte count during induction therapy may provide prognostic information in centers where minimal residual disease detection is not feasible<sup>21</sup>.

## CONCLUSIONS

In the last two decades in our center, development in diagnostic procedures such as immunophenotyping and molecular biology tests at diagnosis and improvements in the supportive care have been made in the care of children with ALL so the OS reached 72% and the 5-year survival rate 67,65%. Further improvements are needed to improve the survival rate of children with ALL: MRD monitoring, access to HSCT in due time, improvement of supportive care.

**Conflict of interests:** none declared.

### Authors contribution

**Conception and design:** Adrienne Horvath, Zsuzsanna Erzsebet Papp

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## Abbreviation

ALL	Acute Lymphoblastic Leukemia
WBC	White blood cells
CNS	Central nervous system
FAB	French-American-British
BM	Bone marrow
BFM	Berlin-Frankfurt-Munster
CSF	Cerebrospinal fluid
SR	Standard risk
MR	Medium risk
HR	High risk
OS	Overall survival
PGR	Prednisone good responders
PPR	Prednisone poor responders
HSCT	Hematopoietic stem cell transplantation
ASCT	Allogeneic stem cell transplantation
AuSCT	Autologous stem cell transplantation
MUD	Matched unrelated donor
MRD	Minimal residual disease
HIC	High-income countries
LMIC	Low and middle-income countries
EFS	Event-free survival

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